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Repetitive sequences: the hidden diversity of heterochromatin in prochilodontid fish

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Abstract

The structure and organization of repetitive elements in fish genomes are still relatively poorly understood, although most of these elements are believed to be located in heterochromatic regions. Repetitive elements are considered essential in evolutionary processes as hotspots for mutations and chromosomal rearrangements, among other functions – thus providing new genomic alternatives and regulatory sites for gene expression. The present study sought to characterize repetitive DNA sequences in the genomes of *Semaprochilodus insignis* (Jardine & Schomburgk, 1841) and *Semaprochilodus taeniurus* (Valenciennes, 1817) and identify regions of conserved syntenic blocks in this genome fraction of three species of Prochilodontidae (*S. insignis*, *S. taeniurus*, and *Prochilodus lineatus* (Valenciennes, 1836) by cross-FISH using *Cot-1* DNA (renaturation kinetics) probes. We found that the repetitive fractions of the genomes of *S. insignis*

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and *S. taeniurus* have significant amounts of conserved syntenic blocks in hybridization sites, but with low degrees of similarity between them and the genome of *P. lineatus*, especially in relation to B chromosomes. The cloning and sequencing of the repetitive genomic elements of *S. insignis* and *S. taeniurus* using *Cot-1* DNA identified 48 fragments that displayed high similarity with repetitive sequences deposited in public DNA databases and classified as microsatellites, transposons, and retrotransposons. The repetitive fractions of the *S. insignis* and *S. taeniurus* genomes exhibited high degrees of conserved syntenic blocks in terms of both the structures and locations of hybridization sites, but a low degree of similarity with the syntenic blocks of the *P. lineatus* genome. Future comparative analyses of other prochilodontidae species will be needed to advance our understanding of the organization and evolution of the genomes in this group of fish.

Keywords

Chromosomal painting, Fish, microsatellites, repetitive sequences, sex chromosome, transposable elements

Introduction

Multiple copies of DNA sequences, known as "repetitive DNA", compose large portions of eukaryotic genomes. Repetitive DNA is generally divided into two groups: (1) tandem repeats, which include DNA satellites, minisatellites, and microsatellites; and (2) dispersed interspersed repeats composed of transposable elements (TEs) (Timberlake 1978, Charlesworth 1994, Jurka 2005), but there are other gene families with sequence repetitions also known as repetitive DNA, such as the genes encoding for ribosomal RNA (rRNA) (Long 1980). While the structure and organization of this genome fraction is still poorly understood in fish, most of these non-coding repetitive sequences appear to be located in heterochromatic regions (Fishcher et al. 2004, Martins et al. 2011).

The repetitive sequences were largely considered to be "junk", "selfish", or "parasitic" DNA (Doolittle and Sapienza 1980, Orgel and Crick 1980, Nowak 1994) due to the lack of any known functions in the genome for these sequences. With ever-increasing volumes of genomic information, however, these repetitive sequences are now known to play larger roles in the structural and functional evolution of the genome (Shapiro and Vonsternberg 2005, Biémont and Vieira 2006). Indeed, repetitive sequences are now known to be involved in chromosomal rearrangements and responsible for significant proportions of the karyotypic variations observed in many groups (Kidwell 2002, Schneider et al. 2013).

In Prochilodontidae, centromeric heterochromatin regions have been observed in all 54 chromosomes in all of the species analyzed, as well as in the B chromosomes of *Prochilodus lineatus* (Valenciennes, 1836) (Pauls and Bertollo 1980, Oliveira et al. 2003, Hatanaka et al. 2002, Terencio et al. 2012). However, *Semaprochilodus insignis* (Jardine & Schomburgk, 1841) has additional heterochromatic blocks in the terminal regions of the first metacentric pair, while *Semaprochilodus taeniurus* (Valenciennes, 1817) has large bitelomeric markings in metacentric pairs 2 and 3.

The ZZ/ZW sex chromosome system may have originated through an *in cis* process of heterochromatin accumulation that differentiated into the W chromosome – with consequent recombination restrictions starting with the first chromosome pair (Terencio et al. 2012a).

The phylogenetic biogeography of the Prochilodontidae indicates that the family dates back minimally to approximately 12 million years ago, with higher level intrafamilial cladogenic events also dating to at least that time period; these dates are congruent with data from the fossil record for more encompassing groups within the Characiformes (Sivasundar and Bermingham 2001, Castro and Vari 2004). Phylogenies constructed based on morphological (information from osteological and soft anatomical systems) and molecular (ATPase, D-loop, ND4 and COI) characters demonstrates that *Prochilodus* is the sister group to the clade formed by *Ichthyoelephas* plus *Semaprochilodus* (Turner et al. 2004). It is believed that heterochromatic regions play important roles in the differentiation of this fish group, despite the relatively stable karyotypic macrostructures of the Prochilodontidae. The genetic composition of these regions is still only poorly understood, however, and the only firm information available concerns the presence of large amounts of repetitive DNA sequences in the B chromosomes of *P. lineatus* (Camacho and Beukeboom 2000) and in the W sex chromosome of *S. taeniurus* (Terencio et al. 2012b). These sequences were identified and classified as microsatellites, transposons, and retrotransposons in the latter species.

Heterochromatic regions are essential to evolutionary processes because of their ability to propagate and influence genes (Grewal and Jia 2007), and the present study therefore sought to characterize the moderate to highly repetitive DNA sequences in *S. insignis* and *S. taeniurus* by cloning and sequencing them and identifying conserved syntenic blocks of this fraction in three species of the family Prochilodontidae (*S. insignis*, *S. taeniurus*, and *P. lineatus*) using cross-FISH techniques with *Cot*-1 DNA probes.

Materials and methods

Ten specimens of *S. insignis* (six females and four males) and 12 *S. taeniurus* (seven females and five males) were examined cytogenetically. These fish were captured with the authorization of ICMBio SISBIO 10609-1/2007 at the confluence of the Negro and Solimões Rivers (AM) and at the Amazonas and Tapajós (PA) Rivers. Five *P. lineatus* (two females and three males) were captured from the Tibagi River (PR). The fish were anesthetized in ice-cold water and were sacrificed. Voucher specimens were deposited in the INPA Animal Genetics Laboratory fish collection (10034, 10037, 10047 and 10696). Chromosome preparations were obtained from anterior kidney cells using an *in vivo* colchicine treatment (Bertollo et al. 1978). Institutional abbrevoations: UFAM, Federal University of Amazonas; INPA, National Institute of Amazonian Research; UEPG, State University of Ponta Grossa.

Isolation of repetitive DNA via re-association kinetics

Enriched samples containing repetitive DNA sequences from *S. insignis* and *S. taeniurus* were constructed based on the renaturation kinetics of *Cot-1* DNA (DNA enriched for highly and moderately repetitive DNA sequences) according to the protocol described by Zwick et al. 2010) and recently adapted by Ferreira and Martins (2008). DNA samples (50 ⊠l of 100-500 ng/⊠l of DNA in 0.3 M NaCl) were autoclaved (121 °C) for 5 minutes (min) to obtain fragments ranging from 100 to 2000 base pairs. Next, the DNA was denatured at 95 °C for 10 min, placed on ice for 10 seconds (s) and subsequently placed at 65 °C for 1 min for re-annealing. The samples were incubated at 37 °C for 8 min with 1 U of S1 nuclease to permit the digestion of single-stranded DNA. The repetitive portion of this DNA was recovered by freezing in liquid nitrogen, and the DNA was extracted using phenol-chloroform. The resulting DNA fragments were used as probes for fluorescence *in situ* hybridization, cloned and sequenced.

Fluorescence *in situ* hybridization (FISH)

The repetitive *S. taeniurus* and *S. insignis* sequence probes isolated using *Cot-1* DNA were labeled with digoxigenin-11-dUTP and biotin-16-dUTP (Dig-Nick Translation mix and Biotin-Nick Translation mix; Roche), respectively, by nick translation reactions following the manufacturer's instructions. Two antibodies, namely, anti-digoxigenin-rhodamine and streptavidin (Life Technologies), were used for signal detection. Fluorescence *in situ* hybridization (FISH) was performed on mitotic chromosome spreads (Pinkel et al. 1986). Homologous and heterologous *in situ* fluorescent hybridizations were performed using 77% stringency (2.5 ng/μl of DNA, 50% deionized formamide, 10% dextran sulfate and 2 × SSC at 37 °C for 18 hours). The chromosomes were counterstained with DAPI (2 μg/ml) in Vectashield mounting medium (Vector).

Microscopy/Image Processing

Hybridized chromosomes were analyzed using an Olympus BX51 epifluorescence microscope, and the images were captured with a digital camera (Olympus DP71) using the Image-Pro MC 6.3 software.

Cloning and sequencing of repetitive sequence

One microgram of the *Cot-1* DNA products was cloned using a pMOS Blunt-ended PCR Cloning Kit (GE Healthcare), purified using the GFX PCR Purification Kit (GE

Healthcare) and sequenced using the Big Dye Kit (Applied Biosystems) in an ABI 3130 genetic analyzer. Sequence alignment was performed using Clustal W (Thompson et al. 1994), which is included in the BioEdit 7.0 software program (Hall 1999). Each clone was used as a query in BLASTN (Basic Local Alignment Search Tool nucleotide) searches against the NCBI nucleotide collection (http://www.ncbi.nlm.nih.gov) and in searches against the Repbase database (Jurka et al. 2005) at the Genetic Information Research Institute (Giri) (http://www.girinst.org/repbase/) using CENSOR software (Kohany et al. 2006).

Results

Hybridization of the *S. insignis Cot-1* DNA probe to its own chromosomes demonstrated that the repetitive elements of its genome were located in the centromeric regions of all chromosomes, as well as in the terminal region of several chromosomes (Fig. 1a and b). The cross-hybridization of the *S. taeniurus Cot-1* DNA probe to the chromosomes of *S. insignis* revealed markers in the centromeric region, although they were smaller than those observed using species-specific probes (Fig. 1c). Additionally, no terminal markers were observed in *S. insignis* using the heterologous probe, indicating that this species has chromosome pairs (Fig. 1d, arrowheads) that carry species-specific repetitive sequences not shared with *S. taeniurus* (Fig. 1a, b, c and d).

Hybridization of the *S. taeniurus Cot-1* DNA probe to its own chromosomes likewise revealed that repetitive sequences were abundant in the genome of this species and located in various regions (e.g., centromeric, interstitial, and terminal) of the entire chromosome complement (Fig. 1e and g). Cross-FISH reactions were performed using the *S. insignis Cot-1* DNA probe and demonstrated the presence of conserved syntenic blocks in several chromosomal regions (Fig. 1f). *S. taeniurus* also displayed species-specific repetitive DNA sites located in the centromeric and terminal regions of 14 chromosomes (Fig. 1h, arrows), with no observed hybridizations of the *S. insignis Cot-1* DNA probe to these same regions (Fig. 1e, f, g, and h).

Both *Cot-1* DNA probes of *S. insignis* and *S. taeniurus* displayed positive hybridization signals in the terminal regions of the entire complement of *P. lineatus* chromosomes. The supernumerary (i.e., B) chromosomes (Fig. l, arrowheads) revealed hybridization signals only with the *S. taeniurus Cot-1* DNA probe. The same marker pattern seen on one of the B chromosomes was also observed on the autosomal chromosomes, while only one of the chromosome arms exhibited hybridization signals shared with the other B chromosome (Fig. 1 i, j, k and l).

Cloning and sequencing the repetitive genome elements obtained from *S. insignis* and *S. taeniurus Cot-1* DNA identified 48 DNA fragments of varying sizes (GenBank: JX848379–JX848393). 71% of repetitive DNA diversity sampled (*Cot*-1 DNA) of *S. insignis* displayed high similarity to microsatellites, 17% to DNA transposons, and

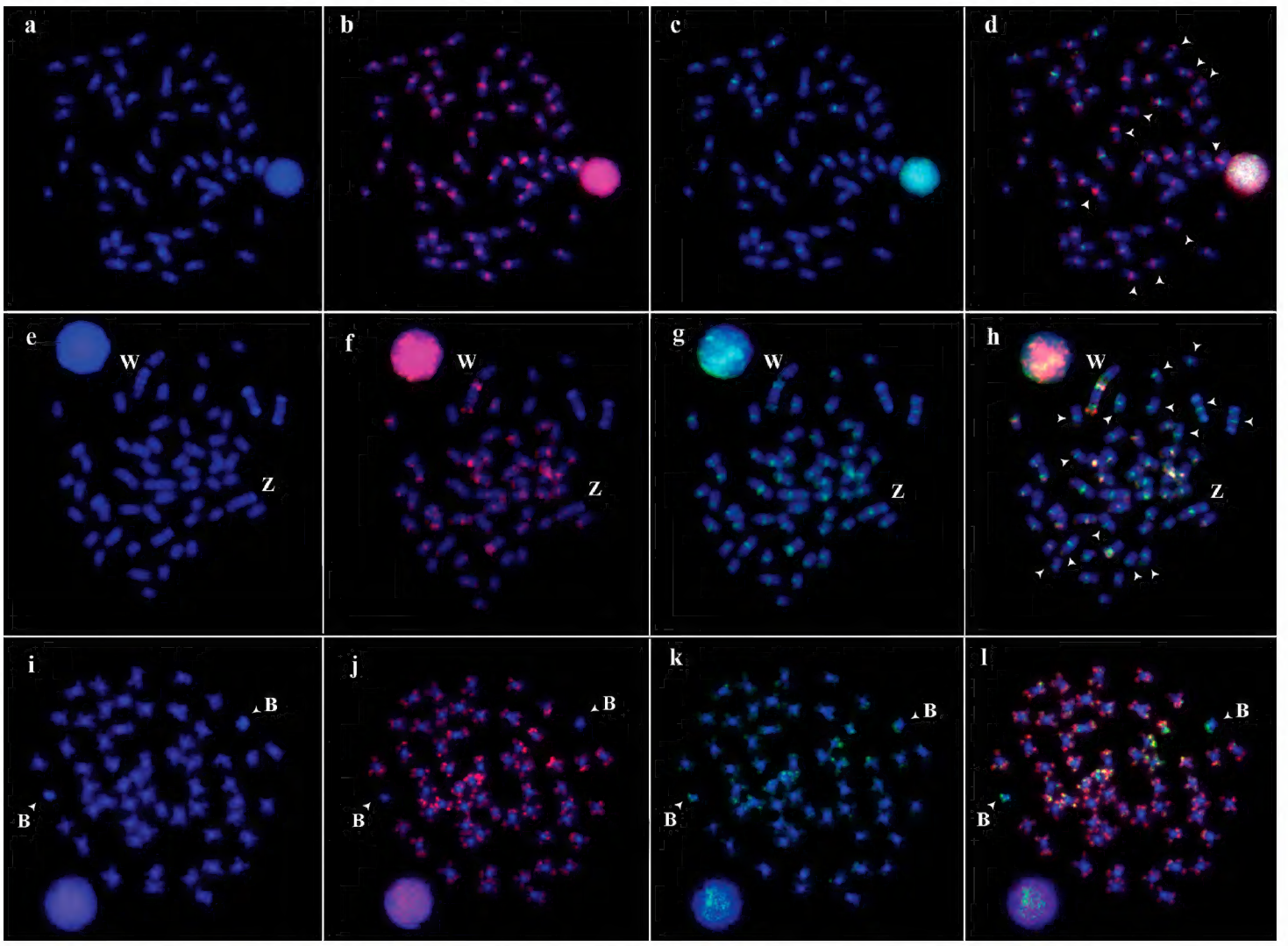

Figure 1. *Cot-1* DNA fraction hybridization in three species of Prochilodontidae. **a** *S. insignis* chromosomes counterstained with DAPI **b** *Cot-1* DNA from the *S. insignis* genome hybridized to its own chromosomes **c** *Cot-1* DNA from the *S. taeniurus* genome hybridized to *S. insignis* chromosomes **d** Double-FISH of the *Cot-1* DNA fraction **e** *S. taeniurus* chromosomes counterstained with DAPI **f** *Cot-1* DNA from the *S. taeniurus* genome hybridized to its own chromosomes **g** *Cot-1* DNA from the *S. insignis* genome hybridized to the *S. taeniurus* chromosomes **h** Double-FISH of the *Cot-1* DNA fraction **i** *P. lineatus* chromosomes counterstained with DAPI **j** *Cot-1* DNA from the *S. insiginis* genome hybridized to *P. lineatus* chromosomes **k** *Cot-1* DNA from the *S. taeniurus* genome hybridized to *P. lineatus* chromosomes **l** Double-FISH of the *Cot-1* DNA fraction.

10% to retrotransposons (Table 1); 75% of the repetitive sequences sampled of *S. taeniurus* displayed high similarity to microsatellites, 5% to transposons, and 15% to retrotransposons (Table 2).

Discussion

Diversity of repetitive DNAs in the genomes of *S. insignis* and *S. taeniurus*

Recent studies have indicated that repetitive sequences have definitely influenced genome evolution by controlling gene activity and by their involvement in chromosomal rearrangements (Valente et al. 2011). The cloning and sequencing the repetitive ge-

Table 1. Nucleotide homology of the *Cot-1* DNA fraction clones of *Semaprochilodus insignis* to known sequences in public databases. BLASTN results and their respective identities are displayed.

Isolate clone	Repetitive sequences	Similarity	Identities
Sin1	DNA transposon	EnSpm-3_DR (RepBase/GIRI*)	70%
Sin2	Microsatellite	*Cyprinus carpio* (GenBank JN756399.1)	92%
Sin3	Microsatellite	*Hypostomus gymnorhynchus* (GenBank HM545164.1)	83%
Sin4	Non-LTR retrotransposon	HERO-2_DR (RepBase/GIRI*)	78%
Sin5	Microsatellite/Retrotransposon	*Colossoma macropomum* (HM579956.1) SINE_2 (RepBase/GIRI*)	79%–75%
Sin6	DNA transposon	Mariner/Tc1 (RepBase/GIRI*)	76%
Sin7	DNA transposon	ERV2 Endogenous Retrovirus (RepBase/GIRI*)	77%
Sin8	Microsatellite	*Cyprinus carpio* (GenBank JN733372.1)	100%
Sin9	Non-LTR retrotransposon	Rex1 (RepBase/GIRI*)	73%
Sin10	Microsatellite	*Cyprinus carpio* (GenBank JN771242.1)	91%
Sin11	DNA transposon	*Labeo rohita*Tc1-like (GenBank AY083617.1)	77%
Sin12	Microsatellite	*Cyprinus carpio* (GenBank JN761177.1)	100%
Sin13	Microsatellite	*Hippoglossus hippoglossus* (GenBank AJ270780.1)	89%
Sin14	DNA transposon	Helitron-2_DR (RepBase/GIRI*)	83%
Sin15	Microsatellite	*Cyprinus carpio* (GenBank JN785563.1)	83%
Sin16	Microsatellite	*Salmo salar* CAG-repeat (GenBank Y11457.1)	87%
Sin17	Microsatellite	*Eleutheronema tetradactylum* (GenBank AB697177.1)	80%
Sin18	Microsatellite	*Oncorhynchus mykiss* (GenBank AY039630.1)	86%
Sin20	Microsatellite	*Prochilodus lineatus* (GenBank AY285824.1)	84%
Sin21	Microsatellite	*Cyprinus carpio* (GenBank JN745523.1)	89%
Sin22	Microsatellite	*Cyprinus carpio* (GenBank JN757227.1)	90%
Sin23	Microsatellite	*Cyprinus carpio* (GenBank JN737559.1)	92%
Sin38	Microsatellite	*Cyprinus carpio* (GenBank JN755429.1)	100%
Sin39	Microsatellite	*Cyprinus carpio* (GenBank JN744936.1)	92%
Sin41	Microsatellite	*Cyprinus carpio* (GenBank JN746351.1)	95%
Sin42	Microsatellite	*Cyprinus carpio* (GenBank JN757934.1)	81%
Sin48	Microsatellite	*Cyprinus carpio* (GenBank JN731077.1)	70%

* Database Repbase (http://www.girinst.org)

nome elements obtained from *S. insignis* and *S. taeniurus Cot-1* DNA displayed high similarities to repetitive sequences deposited in public DNA databases and classified as microsatellites, transposons, and retrotransposons.

Although some repetitive sequences are shared between the two *Semaprochilodus* species analyzed here, DNA sequencing indicated that the genomes of *S. insignis* and *S. taeniurus* were composed of different classes of repetitive sequences. Most of the clones displayed high similarity to microsatellites known from fish species in the order Characiformes (*Colossoma macropomum* Cuvier, 1816) and the family Prochilodontidae (*Prochilodus mariae* Eigenmann, 1922). We believed that the microsatellites were more abundant in this analysis because the method used to obtain the repetitive fraction of

Table 2. Nucleotide homology of the *Cot-1* DNA fraction clones of *Semaprochilodus taeniurus* to known sequences in public databases. BLASTN results along with their respective identities are displayed.

Isolate clone	Repetitive sequences	Similarity	Identities
Ste1	Microsatellite	*Prochilodus mariae* (GenBank JF832400.1)	87%
Ste2	Microsatellite	*Epinephelus fuscoguttatus* (GenBank GU799242.1)	82%
Ste3	Microsatellite	*Cyprinus carpio* (GenBank JN779618.1)	96%
Ste4	DNA transposon	Tc1_FR2(RepBase/GIRI*)	82%
Ste5	Microsatellite	*Cyprinus carpio* (GenBank JN756719.1)	87%
Ste6	Microsatellite	*Cynoglossus semilaevis* (GenBank EU907150.1)	96%
Ste7	Microsatellite	*Prochilodus mariae* (GenBank JF832400.1)	84%
Ste8	Non-LTR retrotransposon	L2-2_DRe (RepBase/GIRI*)	86%
Ste9	Microsatellite	*Cyprinus carpio* (GenBank JN21488.1)	96%
Ste10	Microsatellite	*Cyprinus carpio* (GenBank JN731879.1)	100%
Ste11	Microsatellite	*Cyprinus carpio* (GenBank JN28181.1)	87%
Ste12	Microsatellite	*Prochilodus mariae* (GenBank JF832400.1)	80%
Ste13	Microsatellite	*Salmo salar* (GenBank AJ402727.1)	100%
Ste14	Microsatellite	*Cyprinus carpio* (GenBank JN80674.1)	95%
Ste15	Microsatellite	*Cyprinus carpio* (GenBank JN721488.1)	96%
Ste16	Adeovirus	Bovine Adenovirus type2	99%
Ste17	Non-LTR retrotransposon	SINE3/ 5S (RepBase/GIRI*)	82%
Ste18	Microsatellite	*Brycon amazonicus* (GenBank JQ993450.1)	89%
Ste19	Microsatellite	*Cyprinus carpio* (GenBank JN759566.1)	87%
Ste20	Non-LTR retrotransposon	Rex1-9_XT (RepBase/GIRI*)	75%
Ste21	Non-LTR retrotransposon	L2	82%

* Database Repbase (http://www.girinst.org)

the genome (renaturation kinetics) generates short fragments of DNA (200–300bp) enabling the identification of microsatellites with full homology.

Microsatellites have been observed in a wide range of organisms and are common and widespread in both prokaryote and eukaryote genomes. Among the functions assigned to microsatellites are their participation in chromatin organization, DNA replication, recombination, and the regulation of gene activities (Martins et al. 2011, Li et al. 2011). In fish species such as *Steindachneridion scripta* (Miranda Ribeiro, 1918) *Rineloricaria latirostris* (Boulenger, 1900), and *Danio rerio* (Hamilton, 1822) these repetitive sequences tend to be clustered in the centromeric and telomeric regions (Shimoda et al. 1999, Vanzela et al. 2002). Future studies aimed at mapping microsatellites within the chromosomes of Prochilodontidae will further our understanding of the roles of those sequences in chromosomal evolution in that group.

A certain proportion of these DNA fragments displayed high degrees of similarity to transposable elements (i.e., both transposons and retrotransposons) (Tables 1 and 2) found in the genomes of fish species such as *Xiphophorus maculates* Gunther, 1866, *Leporinus elongatus* Valenciennes, 1850 and *Oryzias hubbsi* Roberts, 1998 (Volff et al. 2000, Bohne et al. 2012, Marreta et al. 2012, Takehana et al. 2012).

The sequences described in the present study may play an evolutionary role in the genomes of *S. insignis* and *S. taeniurus* as one of the sequences identified in the genome of *S. taeniurus* (Ste17) displayed 82% homology with a retrotransposon called SINE3 identified by (Kapitonov and Jurka 2003) as originating from 5S rRNA. As other species of the Prochilodontidae family have only one pair of chromosomes carrying these ribosomal sites, this information strengthens the hypothesis that the multiple 5S rDNA sites observed in *S. insignis* and *S. taeniurus* are pseudogenes (or repetitive sequences) derived from 5S rDNA (Terencio et al. 2012a). In *Gymnotus paraguensis* (Albert & Crampton, 2003) the multiplication of 5S rDNA gene clusters might has be caused by the involvement of transposable elements because the NTS has high identity (90%) with a Tc1-like transposon (Silva et al. 2011).

We were also able to identify sequences in *S. insignis* that exhibited high similarity with the transposable element Helitron. In maize, this TE seems to continually produce new non autonomous elements responsible for the duplicative insertion of gene segments at new locations and for the unprecedented genomic diversity of this species (Morgante et al. 2005). Intact Helitron elements were identified in the sex-determining region of the sex chromosomes of the platyfish *X. maculatus*, suggesting that TE are still active in the genome of platyfish and related species – where they may have roles in the evolution of sex chromosomes and other genomic regions (Zhou et al. 2003).

Tc1/mariner (isolated from the genomes of *S. insignis* and *S. taeniurus*) are the most widespread superfamily of DNA transposons and can be found in fungi, plants, ciliates, and animals (including nematodes, arthropods, fish, frogs, and humans). Most of the transposon copies isolated from vertebrates are clearly inactive remnants of once active transposons that were inactivated by mutations, but only after successfully colonizing their genomes (Plasterk et al. 2009, Ivics et al. 2006).

The retroelement Rex1 was also detected in the repetitive fraction of the genomes of *S. insignis* and *S. taeniurus*. The Rex family has been widely studied in fish, and a number of different lineages have been described in this group (Volff et al. 2000) where they are known to be scattered or grouped into conspicuous clusters in the chromosomes of Neotropical cichlids (Mazzuchelli and Martins 2009, Gross et al. 2009, Teixeira et al. 2009, Oliveira et al. 1999). These elements display compartmentalized distributions in some autosomes and show clear signals along the full lengths of W chromosomes in *S. taeniurus* (Terencio et al. 2012).

The Line2 element was detected only in the repetitive fraction of the *S. taeniurus* genome. This repetitive sequence may be present in the *S. insignis* genome but simply not sampled in our study, or alternatively, it may have been eliminated from the genome of this species. FISH showed that Line2 sequences are organized in small clusters dispersed over all of the chromosomes of *Oreochromis niloticus* (Linnaeus, 1758), but with higher concentrations near chromosome ends (Oliveira et al. 1999). Line elements in mammals appear clustered in the G-banding regions of the chromosomes, and on the sex chromosomes in some cases (Wichman et al. 1992, Fishcher et al. 2004).

Repetitive DNA organization in chromosomes

Repetitive DNA sequences comprising mostly the heterochromatic portions of the genome were observed using the C-banding technique. Previous studies (Feldberg et al. 1987, Vicari et al. 2006, Terencio et al. 2012b) revealed that this technique revealed that repetitive DNA sequence fractions in the genomes of fish of the family Prochilodontidae are not abundant and are located mainly in the centromeric regions of the chromosomes and, less frequently, in the terminal regions of the long arms of some chromosome pairs. Large heterochromatic blocks can be observed, however, in the supernumerary chromosomes (i.e., the B chromosomes) of *Prochilodus spp*. (Vicari et al. 2006, Voltolin et al. 2011) and in the W sex chromosome of *S. taeniurus* (Feldberg et al. 1987, Terencio et al. 2012a). Fluorescence *in situ* hybridization using species-specific probes of the repetitive fractions of the genomes partially confirmed the heterochromatic pattern demonstrated with the C-banding technique in both *S. insignis* and *S. taeniurus* – and positive signals were detected in the centromeric regions of all of their chromosomes. Markers were also observed in the terminal regions of some chromosomes, confirming that repetitive DNAs are also present in this area, although heterochromatin was not observed. Repetitive sequences located outside of heterochromatic regions are believed to significantly influence genome evolution, particularly by controlling and regulating gene activities, and genome sequencing has frequently revealed short and truncated copies of repetitive sequences in euchromatic genomic regions (Fischer et al. 2002, Biémont and Vieira 2006, Timberlake 1978, Yuan and Wessler 2011, Torres et al. 2011). This observation does not necessarily indicate that these repetitive sequences are constitutively expressed, however, since they tend to be silenced and undergo subsequent molecular deterioration. In other words, these sequences becomes inactive and progressively accumulate mutations, insertions, and deletions at neutral rates until completely losing their identities or become lost in the host genome (Fernández-Medina et al. 2012). The presence of repetitive DNAs in euchromatic regions has been observed in many groups, such as insects (Cabral-de-Mello et al. 2011), fish (Teixeira et al. 2009, Valente et al. 2011), and lizards (Pokorná et al. 2011), and these TEs have acquired structural/regulatory functions so that their accumulation in euchromatic regions may lend advantages to the host genome.

Cross-hybridizations of *S. insignis* and *S. taeniurus* showed patterns similar to those observed in homologous hybridization – which suggests that this portion of the genome has been conserved throughout evolution, perhaps due to a functional role. However, revealed that these species have species-specific centromeric and terminal sites not identified by heterologous hybridization.

Cross-FISH using *S. insignis* and *S. taeniurus Cot-1* DNA probes revealed hybridization signals in the subterminal regions of *P. lineatus*, in contrast to the heterochromatic pattern revealed by the C-banding technique with heterochromatin blocks being primarily observed in the centromeric region (Pauls and Bertollo 1990, Venere et al. 1990, Cavallaro et al. 2000, Artoni et al. 2006, Vicari et al. 2006, Voltolin et al. 2011). These data indicate that the repetitive fraction of centromeric heterochromatin of *P.*

lineatus is different from the other two species examined (*S. insignis* and *S. taeniurus*) and that shared repetitive sequences are located on the subtelomeric portions of their chromosomes. This same pattern was observed in three species of *Prochilodus* using (AATTT)n microsatellite (Hatanaka et al. 2002) and W-specific probes (Terencio et al. 2012b).

A common karyotypic feature of species belonging to the genus *Prochilodus* is the presence of supernumerary chromosomes (B chromosomes). Many studies of B chromosomes have indicated that these supernumerary chromosomes are rich in repetitive sequences and, in certain cases, may contain a number of functional genes (Camacho 2005, Ruiz-Estévez et al. 2012). The *P. lineatus* population analyzed in the present study displayed two B chromosomes with distinct hybridization sites. The *S. insignis Cot-1* DNA probe did not hybridize to the B chromosomes, possibly because the repetitive fraction of the *S. insignis* genome is not shared with the B chromosomes of *P. lineatus*. One possible hypothesis explaining this result would be that these repetitive sequences have undergone rapid differentiation and evolution in the genome of *S. insignis*, resulting in a loss of homology with sequences on the B chromosomes of *P. lineatus*. Another explanation could be due to the fact it is different genera, and therefore the Bs found in each species could have different origins. The *S. taeniurus Cot-1* DNA probe was positive, revealing sequence sharing with *P. lineatus* B chromosomes. A number of studies have suggested that B chromosomes can influence sex determination in fish (Noleto et al. 2012, Yoshida et al. 2012), although no relationship between the occurrence of B chromosomes and sex determination has been observed in *P. lineatus*. The B chromosomes detected in *P. lineatus* were recently shown to demonstrate positive signals when hybridized with the W-specific probe, indicating the sharing of repetitive DNA families between these two species (Terencio et al. 2012a).

Conclusions

Results from DNA sequencing indicated that the genomes of *S. insignis* and *S. taeniurus* comprise different classes of repetitive sequences that may have played important roles in their evolution. The repetitive fractions of the *S. insignis* and *S. taeniurus* genomes also exhibit high degrees of conserved syntenic blocks in terms of both the structure and location of hybridization sites. However, the genomes of both *S. insignis* and *S. taeniurus* displayed a low degree of syntenic blocks with the *P. lineatus* genome, especially with regard to the B chromosome, and the origin of this situation has not yet been elucidated.

Authors' contributions

MLT, CHS and MCG collected the samples, collaborated on all cytogenetic procedures, undertook the bibliographic review and coordinated the writing of the manuscript.

EJC, VN, RFA, MCA and MRV participated in the development of the laboratory techniques, performed the specific W-probe for *Semaprochilodus* and reviewed the manuscript. EF coordinated the study and reviewed the manuscript. All authors read and approved of the final manuscript.

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